

Mutagenic Evaluation of Compound FDA 73-23 (Ammonium Alginate) 6/30/75

JW

LBI PROJECT #2468

MUTAGENIC EVALUATION OF

COMPOUND FDA 73-23

PM9005349

AMMONIUM ALGINATE

SUBMITTED TO

FOOD & DRUG ADMINISTRATION  
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
ROCKVILLE, MARYLAND

SUBMITTED BY

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JUNE 30, 1975



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EVALUATION SUMMARY

Compound FDA 73-23, Ammonium Alginate, did not exhibit genetic activity  
in any of the in vitro microbial assays employed in this evaluation.



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DATE: June 30, 1975

SPONSOR: Food and Drug Administration

SUBJECT: Evaluation of Test Compound PM9005349, Ammonium Alginate  
FDA 73-23

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974
2. Description: Light brown powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains: TA-1535  
TA-1537  
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 $\mu\text{M}$
2. Isocitric acid	49 $\mu\text{M}$
3. Tris buffer, pH 7.4	28 $\mu\text{M}$
4. $\text{MgCl}_2$	1.7 $\mu\text{M}$
5. Tissue homogenate fraction	72 mg



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#### D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

#### E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical<sup>a</sup></u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Nonactivation	Ethyl methanesulfonate	Water or saline	BPS <sup>b</sup> FS <sup>b</sup> FS
	2-Nitrofluorene	Dimethylsulfoxide <sup>c</sup>	
	Quinacrine mustard	Water or saline	
Activation	Dimethylnitrosamine	Water or saline	BPS <sup>b</sup> FS
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	

<sup>a</sup> Concentrations given in the Results Section

<sup>b</sup> BPS = base-pair substitution; FS = frameshift

<sup>c</sup> Previously shown to be non-mutagenic

### III. METHODS

#### A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.



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## B. Plate Tests

In the nonactivation procedure, approximately  $10^9$  cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

## C. Suspension Tests

### 1. Nonactivation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^9$  cells/ml and  $5 \times 10^7$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.



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D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program.



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IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: PM9005349
2. Test solvent: DMSO
3. Solubility of the test compound under treatment conditions:  
Insoluble under treatment conditions
4. Additional comments: Light brown powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: November 13, 1974
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

10.0  
1.0  
0.1  
0.01  
0.001

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Dose</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.155	1.25
1/2 50% Survival	0.310	2.50
50% Survival	0.620	5.00
Plate Tests	0.310	--



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V. SUMMARY OF TEST RESULTS

Plate Tests

- A. Name or code designation of the test compound: PM9005349  
 B. Test date: June 20, 1975  
 C. Concentration of the test compound: 0.31%

Test	Species	Tissue	REVERTANTS/PLATE					
			TA-1535		TA-1537		TA-1538	
1	2	1	2	1	2	1	2	
<u>1. Non-activation</u>								
Solvent Control	---	---	16	15	3	8	8	3
Positive Control <sup>a</sup>	---	---	>10 <sup>4</sup>	>10 <sup>4</sup>	>10 <sup>2</sup>	>10 <sup>2</sup>	204	243
Test Compound	---	---	13	10	4	6	5	11
<u>2. Activation</u>								
Negative Control	---	---	47	44	0	1	2	1
Solvent Control	---	---	21	28	8	9	7	10
Reaction Mixture Control	---	---	10	8	11	9	17	13
Positive Control <sup>b</sup>	Mouse	Liver	>10 <sup>3</sup>	>10 <sup>3</sup>	47	46	278	380
Positive Control		Lung	6	6	2	1	4	6
Positive Control		Testes	6	16	5	5	7	8
Positive Control	Rat	Liver	>10 <sup>3</sup>	>10 <sup>3</sup>	38	47	247	243
Positive Control		Lung	5	8	2	1	6	4
Positive Control		Testes	8	14	3	3	5	8
Positive Control	Monkey	Liver	336	337	34	42	252	256
Positive Control		Lung	4	8	2	2	3	1
Positive Control		Testes	9	15	1	6	4	9
Test Compound	Mouse	Liver	10	4	9	14	3	9
Test Compound		Lung	8	8	6	16	7	8
Test Compound		Testes	11	3	4	11	2	14
Test Compound	Rat	Liver	13	10	3	13	6	6
Test Compound		Lung	9	17	3	18	6	7
Test Compound		Testes	6	21	10	9	8	4
Test Compound	Monkey	Liver	12	9	13	9	14	3
Test Compound		Lung	8	9	12	8	12	10
Test Compound		Testes	11	14	8	7	8	9

a TA-1535 EMS 10 µl/plate  
 TA-1537 QM 20 µg/plate  
 TA-1538 NF 100 µg/plate

b TA-1535 DMNA 50 µM/plate  
 TA-1537 AAF 100 µg/plate  
 TA-1538 AAF 100 µg/plate



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**DATA TABLE TERMS AND ABBREVIATIONS**

<b>ABBREVIATION OR TERM</b>	<b>DEFINITION OR EXPLANATION</b>																		
COMPOUND	Client designated compound number appears in this column.																		
TEST CODES	<table> <tr> <td>NAN</td><td>= Nonactivation: Solvent Control</td></tr> <tr> <td>NAP</td><td>= Nonactivation: Positive Control</td></tr> <tr> <td>NA1</td><td>= Nonactivation: Test Compound Dose 1</td></tr> <tr> <td>NA2, etc.</td><td>= Reflects the other dose level(s)</td></tr> <tr> <td>A+C</td><td>= Negative Chemical Control</td></tr> <tr> <td>A-C</td><td>= Activation: Solvent Control</td></tr> <tr> <td>ACP</td><td>= Activation: Positive Control</td></tr> <tr> <td>ACT</td><td>= Activation: Test Compound</td></tr> <tr> <td>A+T</td><td>= Activation: Tissue Control</td></tr> </table>	NAN	= Nonactivation: Solvent Control	NAP	= Nonactivation: Positive Control	NA1	= Nonactivation: Test Compound Dose 1	NA2, etc.	= Reflects the other dose level(s)	A+C	= Negative Chemical Control	A-C	= Activation: Solvent Control	ACP	= Activation: Positive Control	ACT	= Activation: Test Compound	A+T	= Activation: Tissue Control
NAN	= Nonactivation: Solvent Control																		
NAP	= Nonactivation: Positive Control																		
NA1	= Nonactivation: Test Compound Dose 1																		
NA2, etc.	= Reflects the other dose level(s)																		
A+C	= Negative Chemical Control																		
A-C	= Activation: Solvent Control																		
ACP	= Activation: Positive Control																		
ACT	= Activation: Test Compound																		
A+T	= Activation: Tissue Control																		
	<table> <tr> <td>LI</td><td>= Liver Tissue Activation Fraction</td></tr> <tr> <td>LU</td><td>= Lung Tissue Activation Fraction</td></tr> <tr> <td>KI</td><td>= Kidney Tissue Activation Fraction</td></tr> <tr> <td>TE</td><td>= Testes Tissue Activation Fraction</td></tr> <tr> <td>1,2, etc.</td><td>= Dose Levels</td></tr> </table>	LI	= Liver Tissue Activation Fraction	LU	= Lung Tissue Activation Fraction	KI	= Kidney Tissue Activation Fraction	TE	= Testes Tissue Activation Fraction	1,2, etc.	= Dose Levels								
LI	= Liver Tissue Activation Fraction																		
LU	= Lung Tissue Activation Fraction																		
KI	= Kidney Tissue Activation Fraction																		
TE	= Testes Tissue Activation Fraction																		
1,2, etc.	= Dose Levels																		
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>																		
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $\times 10^6$ ).																		
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $10^0$ ). For strain D4, MUT 1 represents the number of ADE+ convertants.																		
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.																		
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.																		
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.																		
CONTAM	Presence of contamination on any plates.																		



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DATA TABLE TERMS AND ABBREVIATIONS (continued)

<u>ABBREVIATION OR TERM</u>	<u>DEFINITION OR EXPLANATION</u>
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey ( <u>Macaca mulatta</u> )
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/08/75

SPECIES / COMPOUND PM9005349

TEST	ORG	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1537 HIS EX-8	TA1535 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
------	-----	-----------------------	-----------------------	-----------------------	-----------------------	-----------------------	-----------------------

NAN		4.00	5.43	2.21	6.43	2.81	2.08
NAP			68.30	118.15	625.95	132.13	78.27
NA1			5.71	3.10	0.42	0.85	0.43
NA2		5.02	1.13	22.04	11.17	1.75	2.23



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/08/75

SPECIES ICRFLO/MOUSE                    COMPOUND PM9005349

TEST	ORG	TA1538 HIS EX-8	TA1538 HIS EX-8	TA1537 HIS EX-8	TA1535 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	7.69		11.83	6.24	2.89	3.14
ACT	A+T	48.48		7.98	9.72	3.01	5.17
ACT	A-C	1.66	21.57	6.13	3.20	2.05	3.45
ACT	PLI	123.74		16.60	2697.44	7.04	11.83
ACT	PLU	6.77		10.37	5.04	5.64	4.14
ACT	PTE	8.54		9.04	7.33	3.80	3.50
ACT	LI1	16.81	4.44	9.36	11.88	0.95	1.77
ACT	LI2	21.82	4.98	7.20	7.59	2.11	1.86
ACT	LU1	3.03		12.10	5.70	0.80	1.19
ACT	LU2	9.76		6.47	5.64	1.56	1.81
ACT	TE1	5.43		7.19	4.81	2.57	1.93
ACT	TE2	8.44		7.02	6.59	2.21	1.82



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/08/75

SPECIES SPRDAW/RAT

COMPOUND PM9005349

TEST	ORG	TA1538	TA1535	TA1537	0000D4	0000D4
		HIS EX-8	HIS EX-8	HIS EX-8	ADE EX-5	TRY EX-5
ACT	A+C	8.52	13.67	4.54	1.97	1.42
ACT	A+T	5.88	15.45	9.11	2.16	2.87
ACT	A-C	3.73	15.10	5.04	0.98	1.63
ACT	PLI	67.92	114.52	8.84	5.65	11.09
ACT	PLU	5.78	9.66	4.87	1.91	2.80
ACT	PTE	5.02	13.67	5.21	3.58	3.29
ACT	LI1	5.14	21.89	3.44	1.27	1.13
ACT	LI2	5.76	11.80	4.06	2.29	2.58
ACT	LU1	3.67	14.11	11.32	1.99	2.61
ACT	LU2	4.56	11.57	5.27	2.04	3.35
ACT	TE1	3.14	11.86	2.80	0.34	0.79
ACT	TE2	3.54	13.58	6.02	2.09	2.20



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 517103		CONTRACT 22374-2104		PROJECT 02468		DATE - 07/08/75	
COMPOND	TEST ID	ORG	SPECIES SPRDAW/RAT	POPUL	MUT1	MUT2	FREQ1
		CONCENTRATION		EP+4	EP+1	EP-5	FREQ2
A+C	DMN 90 UM/ML	0916	0018	0013	1.97	1.42	CONTAM
A+T	***NO MATCH***	0835	0018	0024	2.16	2.87	1
A-C	SALINE	0923	0009	0015	0.98	1.63	0
ACP	LI	DMN 90 UM/ML	0902	0051	0100	5.65	11.09
ACP	LU	DMN 90 UM/ML	0787	0015	0022	1.91	2.80
ACP	TE	DMN 90 UM/ML	1034	0037	0034	3.58	3.29
PM9005349	ACT	LI1 0025-1 PCT.	0711	0009	0008	1.27	1.13
PM9005349	ACT	LI2 0125-2 PCT.	0699	0016	0018	2.29	2.58
PM9005349	ACT	LU1 0025-1 PCT.	0806	0016	0021	1.99	2.61
PM9005349	ACT	LU2 0125-2 PCT.	0586	0014	0023	2.04	3.35
PM9005349	ACT	TE1 0025-1 PCT.	0884	0003	0007	0.34	0.79
PM9005349	ACT	TE2 0125-2 PCT.	0911	0019	0020	2.09	2.20



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 516804				CONTRACT 22374-2104				PROJECT 02468				DATE - 07/08/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	DETECTOR TA1535	SPECIES	RHECUS/MONKEY		POPUP	MUT1	FREQ1		CONTAM	
A+C		DMN 50 UM/ML		1103	0064			EP+6	EP+0	EP-8		0	
A+T		***NO MATCH***		0859	0094							0	
A-C		SALINE		1171	0063							0	
ACP	LI	DMN 50 UM/ML		0862	0534							0	
ACP	LU	DMN 50 UM/ML		1116	0063							0	
ACP	TE	DMN 50 UM/ML		1097	0073							0	
PM9005349	ACT	L11	0031-2 PCT.	0977	0040							0	
PM9005349	ACT	L12	0155-3 PCT.	1155	0130							0	
PM9005349	ACT	LU1	0031-2 PCT.	1033	0036							0	
PM9005349	ACT	LU2	0155-3 PCT.	1199	0059							0	
PM9005349	ACT	TE1	0031-2 PCT.	1119	0059							0	
PM9005349	ACT	TE2	0155-3 PCT.	1501	0066							0	



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 517101 CONTRACT 22374-2104 DETECTOR TA1537 PROJECT 02468  
SPECIES RHECUS/MONKEY

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUTI	FREQ1	CONTAM
A+C		AAF 800 UG/ML	0890	0080	8.99	0	
A+T		***NO MATCH***	0543	0064	11.79	0	
A-C		DMSO	0501	0053	10.58	0	
ACP	LI	AAF 800 UG/ML	0886	0064	7.22	0	
ACP	LU	AAF 800 UG/ML	0748	0072	9.63	0	
ACP	TE	AAF 800 UG/ML	1287	0071	5.52	0	
PM9005349	ACT	L11 0031-2 PCT.	0614	0035	5.70	0	
PM9005349	ACT	L12 0155-3 PCT.	0610	0052	8.52	2	
PM9005349	ACT	LU1 0031-2 PCT.	1170	0104	8.89	0	
PM9005349	ACT	LU2 0155-3 PCT.	0989	0133	13.45	0	
PM9005349	ACT	TE1 0031-2 PCT.	1287	0059	4.58	0	
PM9005349	ACT	TE2 0155-3 PCT.	0746	0098	13.14	0	



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 516901 CONTRACT 22374-2104 DETECTOR TA1538 PROJECT 02468  
SPECIES RHECUS/MONKEY DATE - 07/08/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
A+C		AAF 800 UG/ML	0186	0019	10.22	EP-8	2
A+T		***NO MATCH***	0192	0018	9.38		1
A-C		DMSO	0212	0007	3.30		1
ACP	L1	AAF 800 UG/ML	0190	0194	102.11		0
ACP	LU	AAF 800 UG/ML	0235	0022	9.36		1
ACP	TE	AAF 800 UG/ML	0222	0023	10.36		1
PM9005349	ACT	L11 0031-2 PCT.	0172	0016	9.30		0
PM9005349	ACT	L12 0155-3 PCT.	0282	0019	6.74		0
PM9005349	ACT	LU1 0031-2 PCT.	0248	0012	4.84		0
PM9005349	ACT	LU2 0155-3 PCT.	0284	0007	2.46		0
PM9005349	ACT	TE1 0031-2 PCT.	0944	0014	1.48		0
PM9005349	ACT	TE2 0155-3 PCT.	0235	0004	1.70		0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 517104 DETECTOR 0000D4 SPECIES RHECUS/MONKEY  
PROJECT 02468 DATE - 07/08/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
A+C		DMN 90 UM/ML	1117	0015	0017	1.34	1.52	0	
A+T		***NO MATCH***	1001	0026	0025	2.60	2.50	4	
A-C		SALINE	0984	0011	0015	1.12	1.52	0	
ACP	LI	DMN 90 UM/ML	1108	0022	0064	1.99	5.78	0	
ACP	LU	DMN 90 UM/ML	0887	0039	0018	4.40	2.03	0	
ACP	TE	DMN 90 UM/ML	1072	0048	0032	4.48	2.99	0	
PM9005349	ACT	LII 0025-1 PCT.	1028	0008	0015	0.78	1.46	4	
PM9005349	ACT	LII 0125-2 PCT.	0979	0025	0020	2.55	2.04	0	
PM9005349	ACT	LII 0025-1 PCT.	0911	0007	0022	0.77	2.41	0	
PM9005349	ACT	LU2 0125-2 PCT.	1025	0017	0019	1.66	1.85	2	
PM9005349	ACT	TE1 0025-1 PCT.	0939	0010	0020	1.06	2.13	0	
PM9005349	ACT	TE2 0125-2 PCT.	0889	0027	0031	3.04	3.49	6	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 516802 CONTRACT 22374-2104 PROJECT 02468  
DETECTOR TA1538 SPECIES SPRDAW/RAT

DATE - 07/08/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
A+C		AAF 800	UG/ML	0223	0019	8.52	0
A+T		***NO MATCH***		0221	0013	5.88	2
A-C		DMSO		0241	0009	3.73	0
ACP	LI	AAF 800	UG/ML	0212	0144	67.92	2
ACP	LU	AAF 800	UG/ML	0346	0020	5.78	2
ACP	TE	AAF 800	UG/ML	0319	0016	5.02	0
PM9005349	ACT	L11	0031-2 PCT.	0214	0011	5.14	0
PM9005349	ACT	L12	0155-3 PCT.	0347	0020	5.76	0
PM9005349	ACT	LU1	0031-2 PCT.	0245	0009	3.67	0
PM9005349	ACT	LU2	0155-3 PCT.	0329	0015	4.56	1
PM9005349	ACT	TE1	0031-2 PCT.	0350	0011	3.14	1
PM9005349	ACT	TE2	0155-3 PCT.	0367	0013	3.54	1



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 517006			CONTRACT 22374-2104			DETECTOR TA1537			SPECIES SPRDAW/RAT			PROJECT 02468		DATE - 07/08/75	
COMPOUND	TEST	ORG ID		CONCENTRATION		POPUL	MUTL		FREQ1					CONTAM	
A+C		AAF 800	UG/ML	1454	0066				EP+6	EP+0				0	
A+T		***NO MATCH***		0626	0057						4•54			0	
- A-C		DMSO		1509	0076						9•11			0	
ACP	L1	AAF 800	UG/ML	0961	0085						5•04			0	
ACP	LU	AAF 800	UG/ML	1521	0074						8•84			0	
ACP	TE	AAF 800	UG/ML	1497	0078						4•87			2	
PM9005349	ACT	L11	0031-2 PCT.	1192	0041						5•21			0	
PM9005349	ACT	L12	0155-3 PCT.	1157	0047						3•44			0	
PM9005349	ACT	LU1	0031-2 PCT.	0574	0065						4•06			2	
PM9005349	ACT	LU2	0155-3 PCT.	1044	0055						11•32			2	
PM9005349	ACT	TE1	0031-2 PCT.	1216	0034						5•27			2	
PM9005349	ACT	TE2	0155-3 PCT.	1047	0063						2•80			0	
											6•02			2	



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	22374-2104	DETECTOR	TA1535	SPECIES	SPRDW/RAT	PROJECT	02468	DATE	-	07/08/75
COMPOUND	TEST	ORG ID	CONCENTRATION		POPU	MUT1	FREQ1		CONTAM		
					EP+6	EP+0	EP-8				
A+C		DMN 50 UM/ML		0907	0124		13.67		2		
A+T		***NO MATCH***		0725	0112		15.45		0		
A-C		SALINE		0881	0133		15.10		2		
ACP	LI	DMN 50 UM/ML		0613	0702		114.52		0		
ACP	LU	DMN 50 UM/ML		1097	0106		9.66		0		
ACP	TE	DMN 50 UM/ML		0929	0127		13.67		0		
PM9005349	ACT	LI1	0031-2 PCT.		0644	0141		21.89		0	
PM9005349	ACT	LI2	0155-3 PCT.		0958	0113		11.80		0	
PM9005349	ACT	LU1	0031-2 PCT.		0659	0093		14.11		0	
PM9005349	ACT	LU2	0155-3 PCT.		0700	0081		11.57		0	
PM9005349	ACT	TE1	0031-2 PCT.		0742	0088		11.86		0	
PM9005349	ACT	TE2	0155-3 PCT.		0751	0102		13.58		0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 516903 DETECTOR 0000D4

PROJECT 02468  
DATE - 07/08/75

COMPOUND	TEST	ORG	ID	CONCENTRATION	POPU	MUT1	MUT2	FREQ1	FREQ2	
					EP+4	EP+1	EP+1	EP-5	EP-5	CONTAM
A+C		DMN	90	UM/ML	0795	0023	0025	2.89	3.14	2
A+T		***NO MATCH***			0831	0025	0043	3.01	5.17	1
A-C		SALINE			0782	0016	0027	2.05	3.45	1
ACP	LI	DMN	90	UM/ML	0938	0066	0111	7.04	11.83	0
ACP	LU	DMN	90	UM/ML	0798	0045	0033	5.64	4.14	0
ACP	TE	DMN	90	UM/ML	0999	0038	0035	3.80	3.50	1
PM9005349	ACT	L11	0025-1	PCT.	0733	0007	0013	0.95	1.77	5
PM9005349	ACT	L12	0125-2	PCT.	0806	0017	0015	2.11	1.86	0
PM9005349	ACT	L11	0025-1	PCT.	0754	0006	0009	0.80	1.19	1
PM9005349	ACT	L12	0125-2	PCT.	0831	0013	0015	1.56	1.81	1
PM9005349	ACT	TE1	0025-1	PCT.	0623	0016	0012	2.57	1.93	1
PM9005349	ACT	TE2	0125-2	PCT.	0770	0017	0014	2.21	1.82	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 517506 DETECTOR TA1538 SPECIES ICRFLO/ MOUSE DATE - 07/08/75

COMPOUND	TEST	ORG	ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
	A-C		DMSO		EP+6	EP+0	EP-8	
PM9005349	ACT	LII	0031-2 PCT.		0343	0074	21.57	0
PM9005349	ACT	LI2	0155-3 PCT.		0293	0013	4.44	2
					0241	0012	4.98	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 516101 DETECTOR TA1538

PROJECT 02468  
DATE - 07/08/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP	MUT1	FREQ1	CONTAM
A+C		AAF 800 UG/ML	0156	0012	7.69	1	
A+T		***NO MATCH***	0132	0064	48.48	2	
A-C		DMSO	0181	0003	1.66	0	
ACP	LI	AAF 800 UG/ML	0198	0245	123.74	3	
ACP	LU	AAF 800 UG/ML	0192	0013	6.77	1	
ACP	TE	AAF 800 UG/ML	0164	0014	8.54	1	
PM9005349	ACT	L11	0031-2 PCT.	0226	0038	16.81	3
PM9005349	ACT	L12	0155-3 PCT.	0220	0048	21.82	2
PM9005349	ACT	LU1	0031-2 PCT.	0264	0008	3.03	1
PM9005349	ACT	LU2	0155-3 PCT.	0164	0016	9.76	1
PM9005349	ACT	TE1	0031-2 PCT.	0258	0014	5.43	1
PM9005349	ACT	TE2	0155-3 PCT.	0225	0019	8.44	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 517804			CONTRACT 22374-2104	DETECTOR TA1537	SPECIES ICRFLO/MOUSE	PROJECT 02468	DATE - 07/08/75
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUP EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
A+C		AAF 800	UG/ML	0727	0086	11.83	0
A+T		*****NO MATCH****		0489	0039	7.98	3
A-C		DMSO		0734	0045	6.13	1
ACP	LI	AAF 800	UG/ML	0940	0156	16.60	2
ACP	LU	AAF 800	UG/ML	0733	0076	10.37	2
ACP	TE	AAF 800	UG/ML	0785	0071	9.04	0
PM9005349	ACT	LI1	0031-2 PCT.	0780	0073	9.36	0
PM9005349	ACT	LI2	0155-3 PCT.	0889	0064	7.20	1
PM9005349	ACT	LU1	0031-2 PCT.	0562	0068	12.10	0
PM9005349	ACT	LU2	0155-3 PCT.	0959	0062	6.47	0
PM9005349	ACT	TE1	0031-2 PCT.	0834	0060	7.19	1
PM9005349	ACT	TE2	0155-3 PCT.	0983	0069	7.02	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 516702 DETECTOR TA1535 PROJECT 02468

DATE - 07/08/75

COMPOUND	TEST	ID	ORG	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
A+C			DMN	50 UM/ML	0849	0053	6.24	2
A+T			*	***NO MATCH***	0391	0038	9.72	0
A-C			SALINE		0813	0026	3.20	0
ACP	LI		DMN	50 UM/ML	0273	7364	2697.44	0
ACP	LU		DMN	50 UM/ML	0674	0034	5.04	0
ACP	TE		DMN	50 UM/ML	0232	0017	7.33	0
PM9005349	ACT	L11	0031-2 PCT.		0589	0070	11.88	2
PM9005349	ACT	L12	0155-3 PCT.		0606	0046	7.59	2
PM9005349	ACT	LU1	0031-2 PCT.		0579	0033	5.70	2
PM9005349	ACT	LU2	0155-3 PCT.		0691	0039	5.64	0
PM9005349	ACT	TE1	0031-2 PCT.		0748	0036	4.81	1
PM9005349	ACT	TE2	0155-3 PCT.		0592	0039	6.59	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 516105			CONTRACT 22374-2104			PROJECT 02468			DATE - 07/08/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	SPECIES	/	POPU	MUT1	MUT2	FREQ1	FREQ2
						EP+4	EP+1	EP+1	EP-5	EP-5
NAN			SALINE			1638	0046	0034	2.81	2.08
NAP			EMS 1.0 %			1192	1575	0933	132.13	78.27
PM9005349	NA1		0025-1 PCT.			1171	0010	0005	0.85	0.43
PM9005349	NA2		0125-2 PCT.			1660	0029	0037	1.75	2.23

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 516104 DETECTOR TA1538

SPECIES /

PROJECT 02468

DATE - 07/08/75

COMPOUND	TEST	ORG	ID	CONCENTRATION	POPU	MUT1	FREQ1	CONTAM
					EP+6	EP+0	EP-8	
NAN		DMSO			0184	0010	5.43	1
NAP		NF 125	UG-ML		0448	0306	68.30	1
PM9005349	NA1		0031-2	PCT.	0280	0016	5.71	0
PM9005349	NA2		0155-3	PCT.	0265	0003	1.13	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT	CONTRACT	TEST	ORG	ID	CONCENTRATION	SPECIES	PROJECT	DATE
517508	22374-2104	DETECTOR	TA1537	/	/	/	02468	- 07/08/75
PM9005349	NA2	NA2	NAN	NAN	SALINE	0155-3 PCT.	0917 0046	0917 0046
							4.00	4.00
							5.02	5.02
							0	0
							CONTAM	CONTAM

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104  
EXPERIMENT 516103 DETECTOR TA1537

COMPOND	TEST	ORG ID	CONCENTRATION	SPECIES	PROJECT 02468 /	DATE - 07/08/75
NAN				POPU	MUT1	FREQ1
NAP				EP+6	EP+0	EP-8
PM9005349	NA1	QM 1.0	UG/ML	0272	0006	CONTAM
PM9005349	NA2	0031-2	PCT.	0303	0358	2.21
		0155-3	PCT.	0645	0020	118.15
				0363	0080	3.10
						1
						0
						22.04



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 516102			CONTRACT 22374-2104			PROJECT 02468			DATE - 07/08/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	SPECIES	/	POPU	MUT1	FREQ1	CONTAM	EP-8
NAN						0856	0055	6.43		0
NAP						0682	4269	625.95		0
PM9005349	NA1		0031-2 PCT.			2606	0011	0.42		0
PM9005349	NA2		0155-3 PCT.			0528	0059	11.17		0

**APPENDIX**  
**Tabulation of Data**



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C. Conclusions

The test compound, Ammonium Alginate, did not exhibit genetic activity in the assays employed in this evaluation.

Submitted by:

David Brusick

David Brusick, Ph.D.  
Director of Genetics



BIONETICS

## VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound PM9005349, Ammonium Alginate, was tested for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

### A. Salmonella typhimurium

#### 1. Plate tests

At a concentration of 0.31%, PM9005349, did not exhibit any mutagenic activity for the bacterial indicator organisms in direct or activation plate tests.

#### 2. Nonactivation suspension tests

The results of these tests were negative. The NA2 dose with TA-1537 was repeated because of an increased mutant frequency. The repeat test was negative.

#### 3. Activation suspension tests

The results of these tests were negative. The LI1 and LI2 doses with TA-1538 using mouse tissue were repeated because of increased mutation frequencies. The repeat tests were negative. The PLI for TA-1537 using rat and monkey tissues was lower than usual and compared to the A+T control did not really demonstrate a positive response. This situation is not completely uncommon since the ability of TA-1537 to respond to AAF is marginal under the best of conditions. Occasionally we fail to see a true increase. The fact that the negative controls were satisfactory and that the activation system was effective for other indicators led to the conclusion that the data were acceptable.

### B. Saccharomyces cerevisiae

#### 1. Nonactivation suspension tests

The results of these tests were negative.

#### 2. Activation suspension tests

The results of these tests were negative.



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